

# **Technical Explanation**

## **US Application 10/583,706**

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# Claim 1 of the present application

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## (TP-FP primer set)

A primer set comprising at least two primers that allows a target nucleic acid sequence to be amplified,

wherein a **first primer** included in the primer set contains, in its 3' end portion, a sequence (Ac') that hybridizes to a sequence (A) located in the 3' end portion of the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Ac'), a sequence (B') that hybridizes to a complementary sequence (Bc) to a sequence (B) that is present on the 5' side with respect to the sequence (A) in the target nucleic acid sequence, and

a **second primer** included in the primer set contains, in its 3' end portion, a sequence (Cc') that hybridizes to a sequence (C) located in the 3' end portion of a complementary sequence to the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Cc'), a folded sequence (D-Dc') that contains, on the same strand, two nucleic acid sequences that hybridize to each other.

\*The first primer is **TP**, the second primer is **FP**.

**TP; Turn-back Primer**

**FP; Folded Primer**

# Technical explanation of the TP and FP

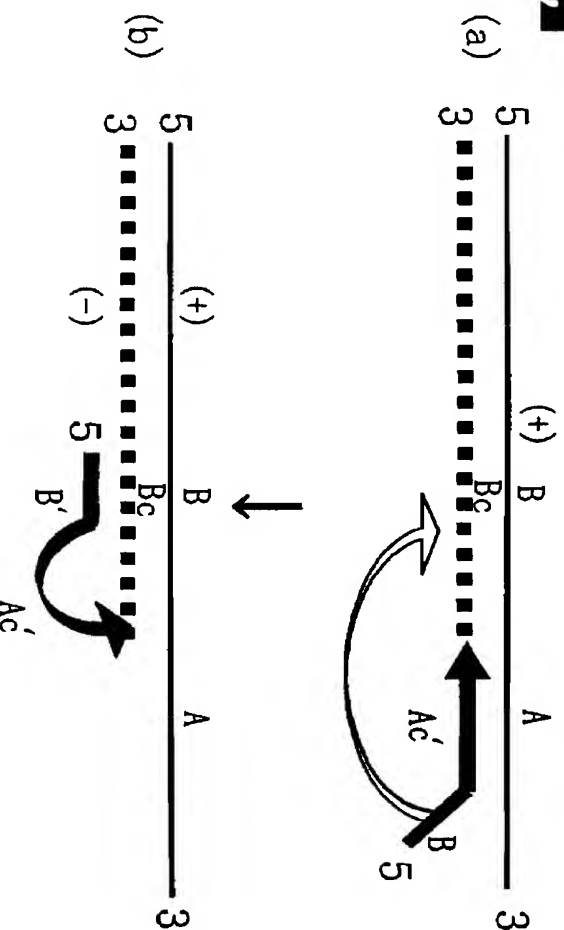


## **FP has the function as follows;**

- (1) FP has the folded sequence (D- (a) Dc') in the 5' side sequence.
- (2) The folded sequence (D-Dc') has two nucleic acid sequences that hybridize to each other.
- (3) The folded sequence (D-Dc') **DO NOT** hybridize to the elongation strand from FP.

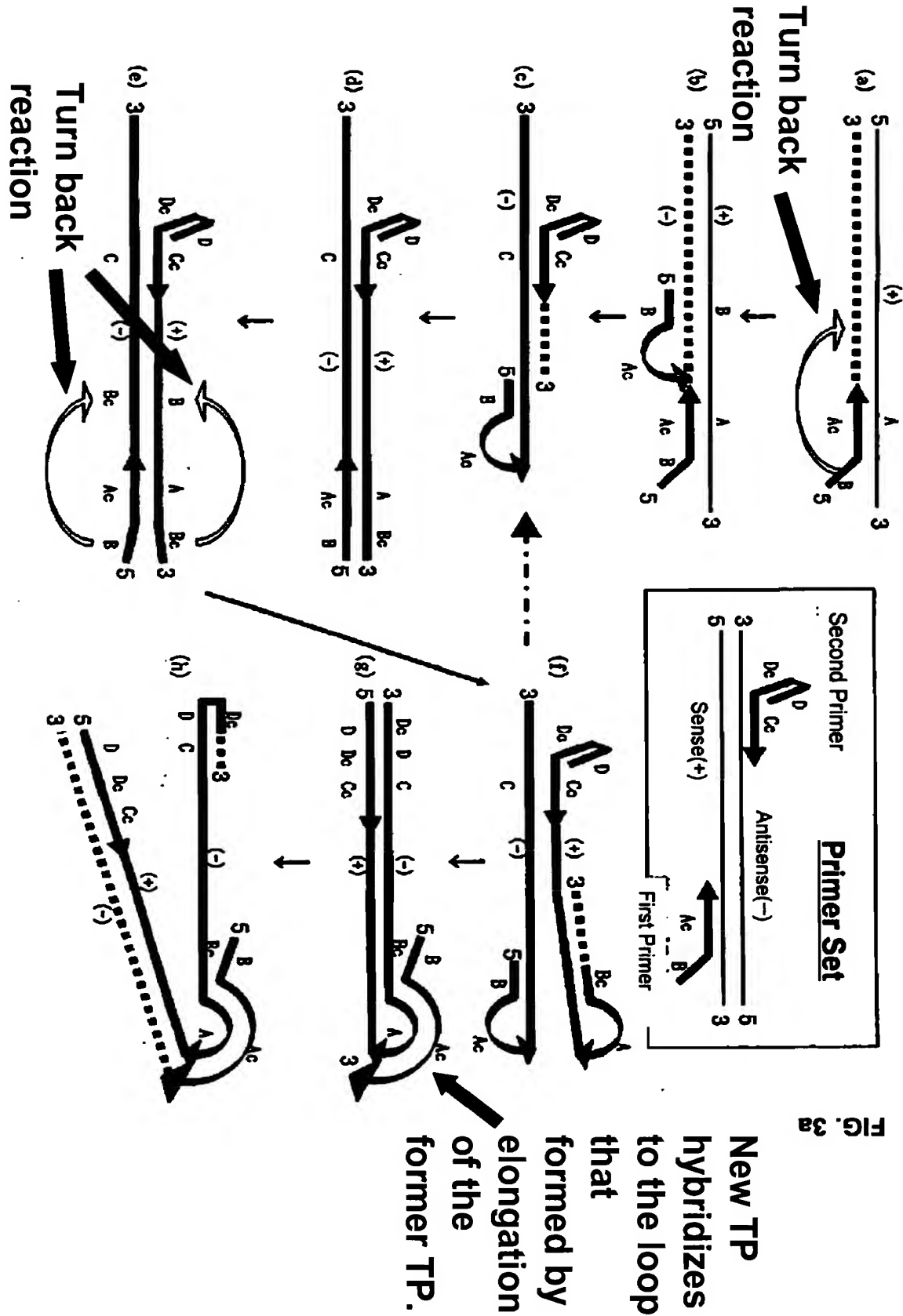
## **TP has the function as follows;**

- (1) TP has the turn back portion (B) in the 5' side sequence.
- (2) The turn back portion (B) can hybridize to the portion (Bc) of the elongation strand from TP.



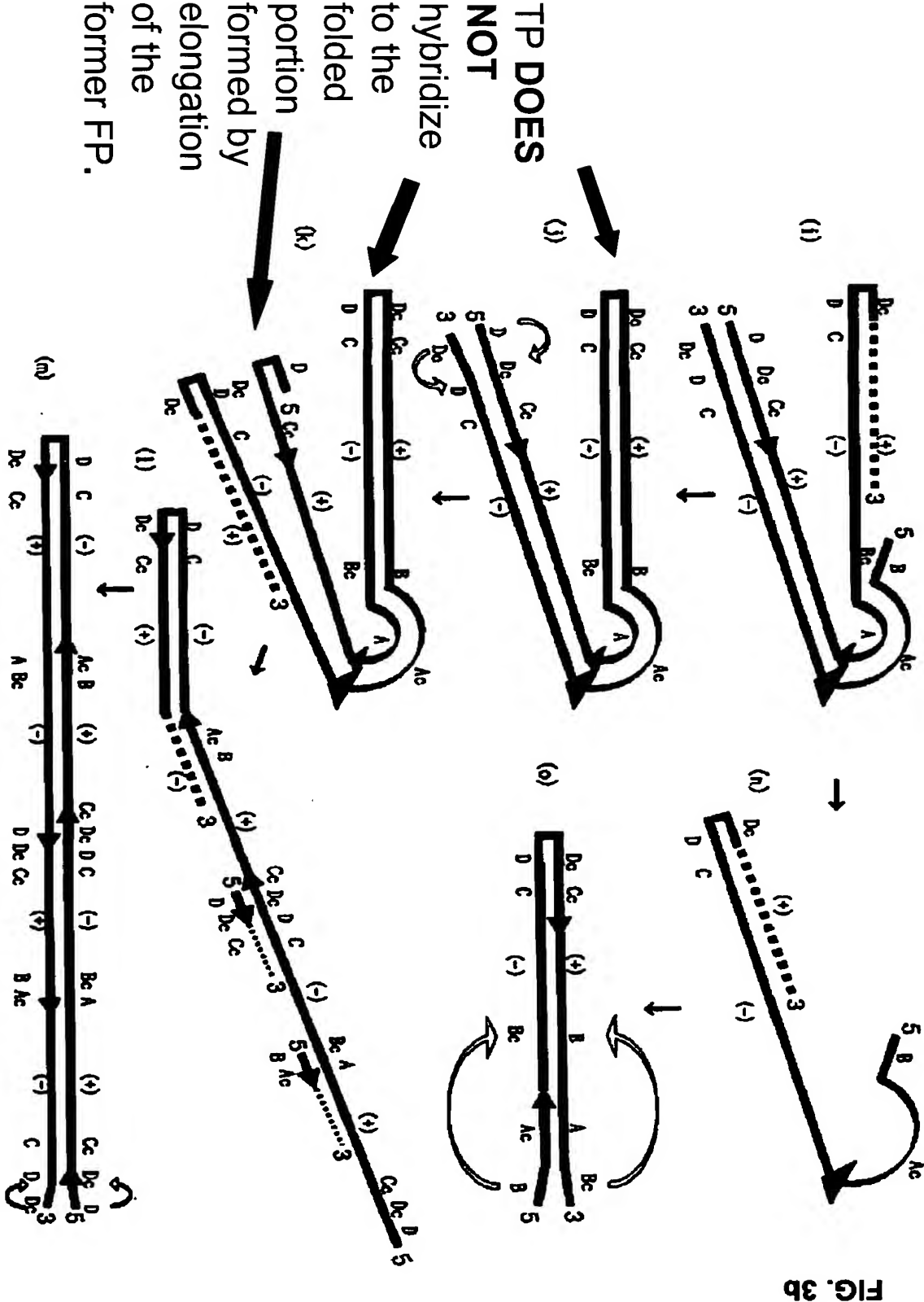
# Mechanism of the amplification reaction of the TP-FP(1)

(FIG.3 of the present invention)



# Mechanism of the amplification reaction of the TP-FP(2)

(FIG.3 of the present invention)



## **The present invention has four advantages.**

### **(1) Isothermal amplification**

- The amplification occurs without thermal denaturation.

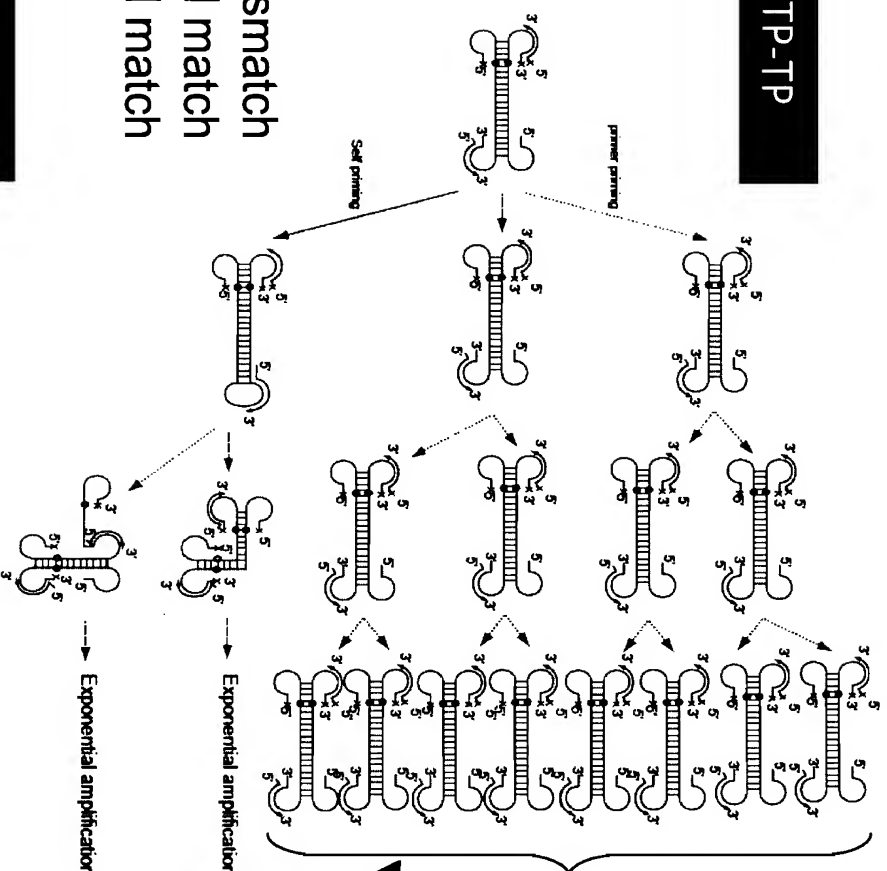
### **(2) Specific amplification**

- The present invention can detect SNPs without non-specific amplification.

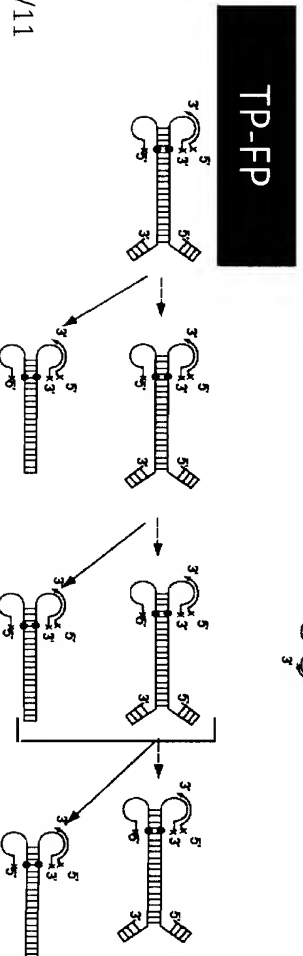
### **(3) Short time amplification**

### **(4) Easy primer design**

TP-TP



- ## Exponential amplification



- 2010/8/11

# Short time amplification and easy primer design(1)

## (1) TP

- (i) TP can amplify exponentially.
- (ii) TP has a strong engine of amplification.
- (iii) TP has two area depending on template sequence.

## (2) FP

- (i) FP can not amplify exponentially, but amplify linearly.
- (ii) FP is like a mirror which reflect TP amplification.
- (iii) FP needs only one area depending on template sequence.

## (3) TP-TP primer set

- (i) TP-TP primer set needs four areas depending on the template sequence.
- (ii) TP-TP Primer set needs a design of a couple of good TP because the reaction is totally controlled by no good TP.
- (iii) TP-TP Primer set is difficult to design.

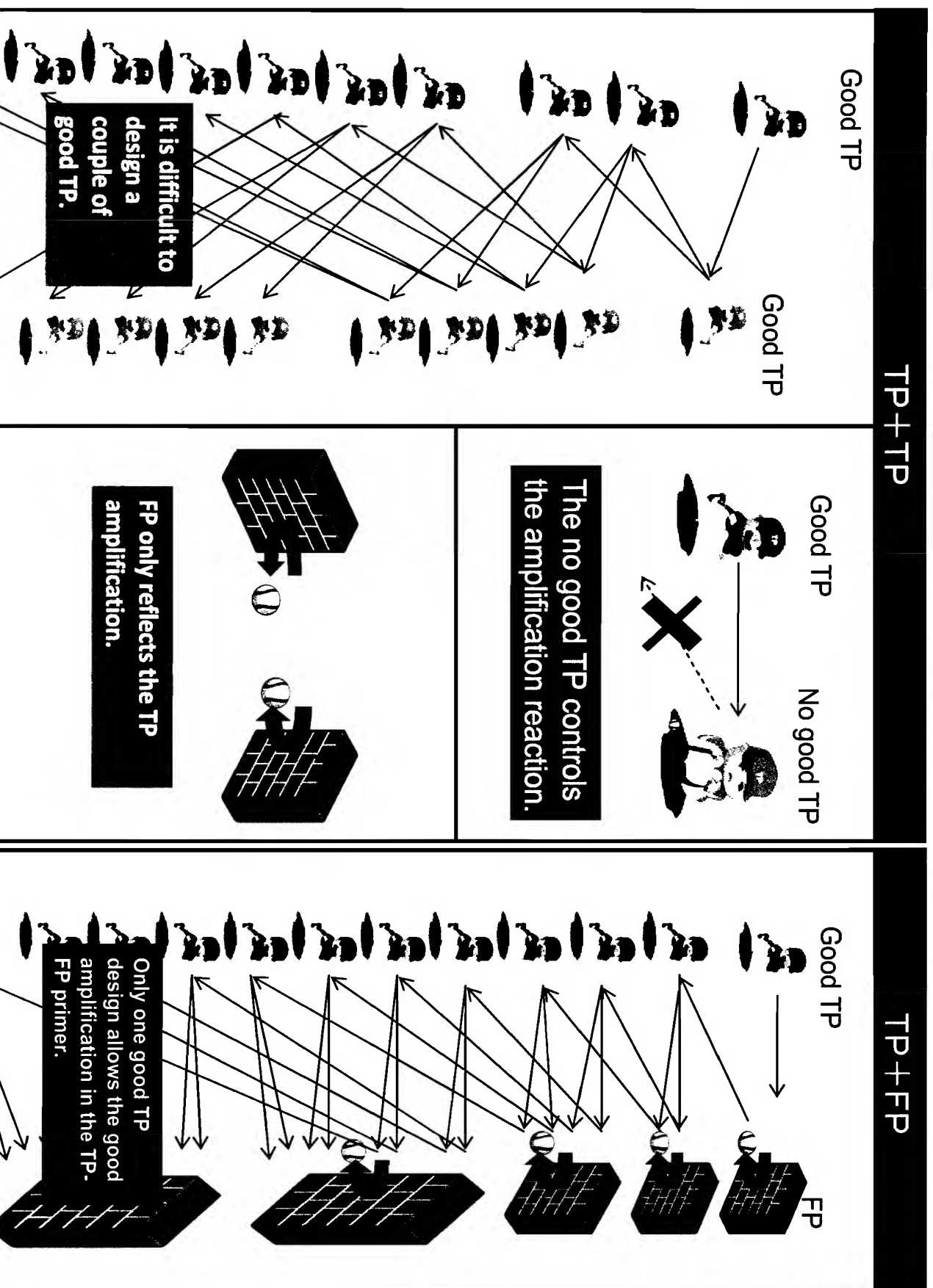
## (4) TP-FP primer set

- (i) TP-FP primer set needs only three areas depending on the template sequence.
- (ii) TP-FP Primer set needs a design of only one good TP because FP whose folded sequence can be designed in advance independently from template sequence **DOES NOT** control the reaction.
- (iii) TP-FP Primer set is easy to design.



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## Short time amplification and easy primer design(2)



# Office Action (1)

## · Summary of the Office Action

The examiner pointed out as follows;

- (1) TP are shown in Figure 4, step 1 and 2 (① and ② shown in below left) in Rabbani (EP0971039A2).
- (2) FP are shown in Figure 1, step 3 (③ shown in below right) in Rabbani.
- (3) Therefore, Claims 1 to 5 of the present invention lacks novelty (102(b)).

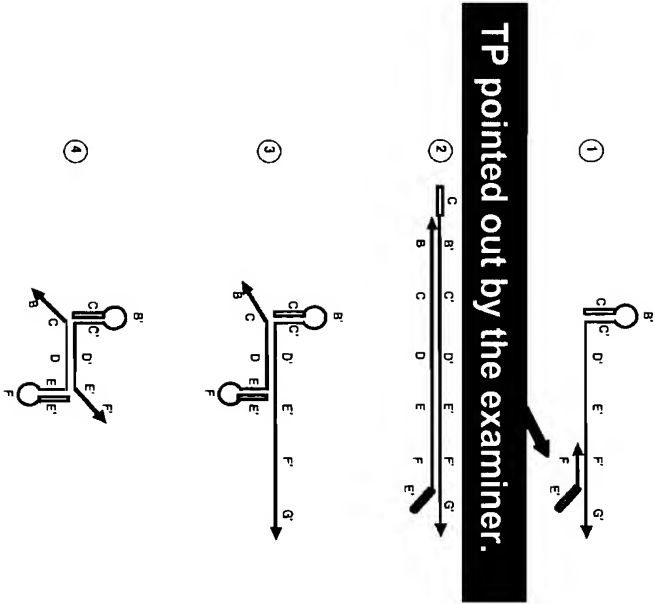


FIGURE 4

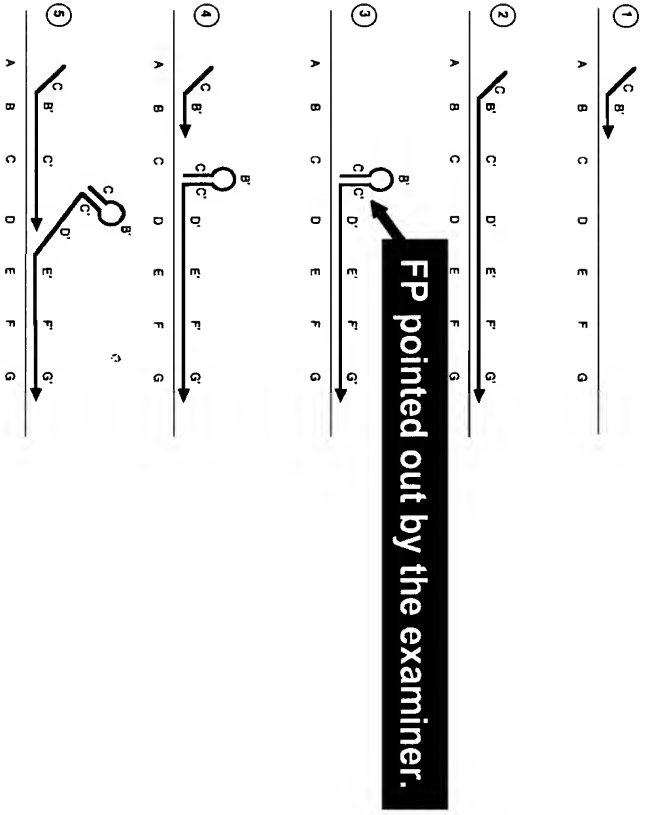


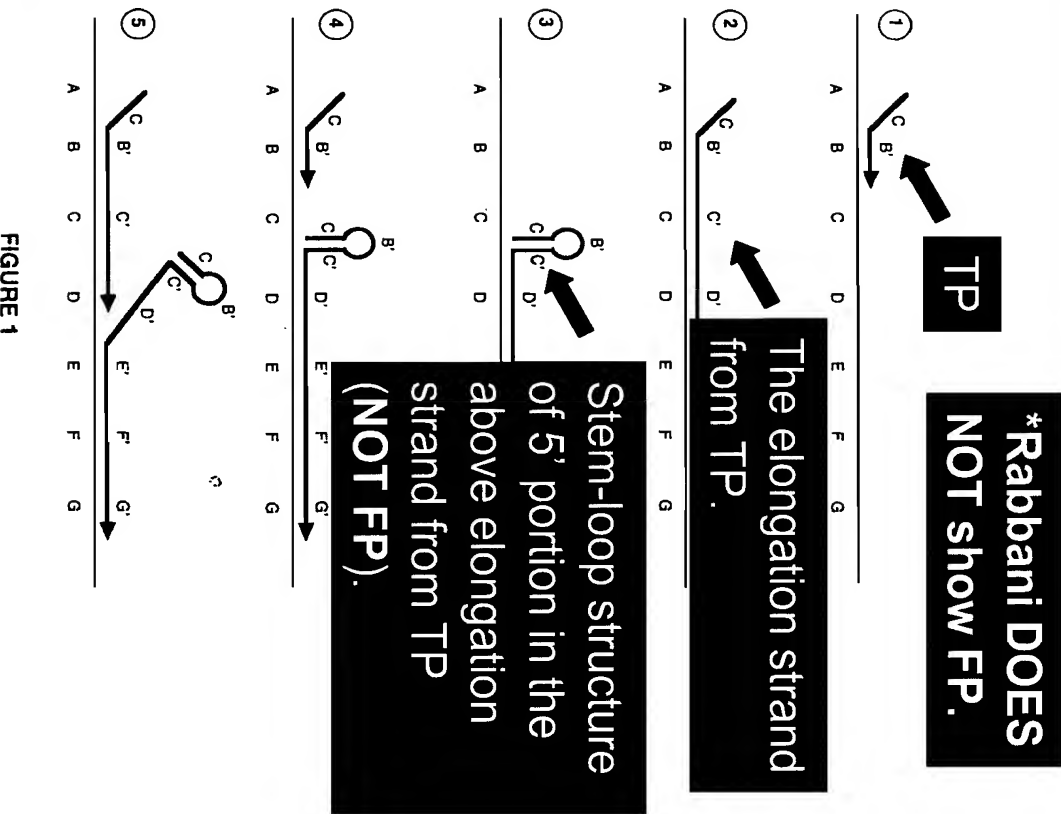
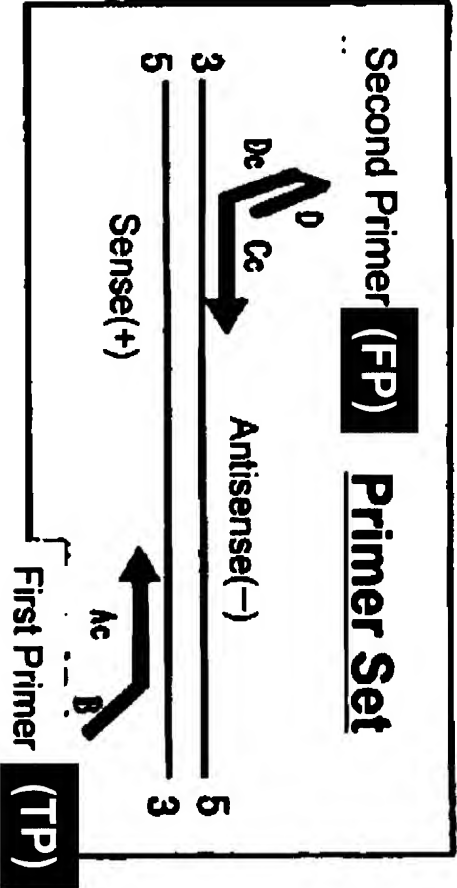
FIGURE 1

**Office Action (2); FIGURE 1 ③ in Rabbani is NOT FP.**

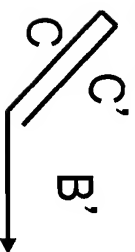
(1) FIGURE 1 ③ in Rabbani shows the elongation strand from TP.

(2) **Primer is different from the elongation strand.**

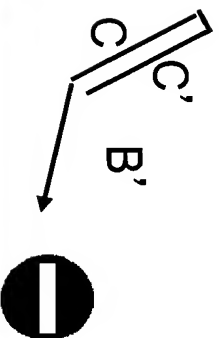
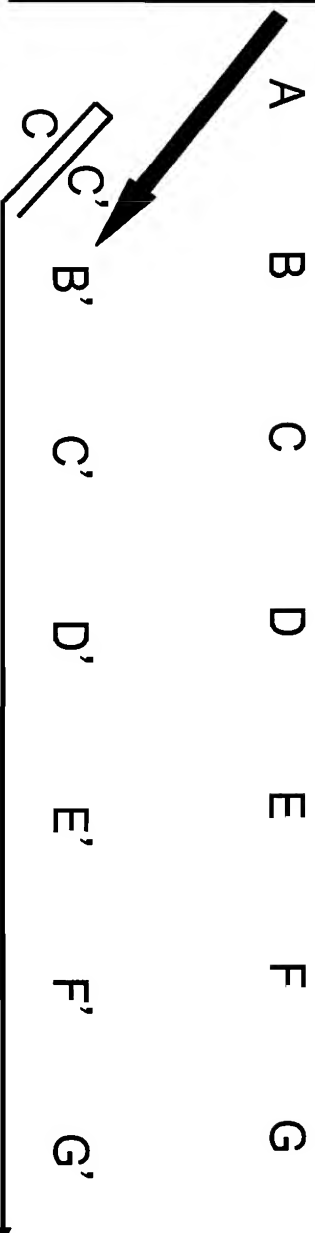
(3) Rabbani **DOES NOT** show the TP-FP primer set of the present invention.



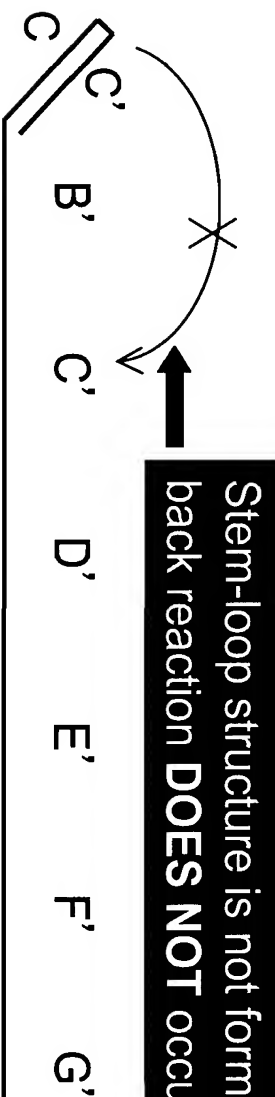
# In case FP is used in the FIGURE 1 in Rabbani.



The sequence B'  
**DOES NOT** form  
stem-loop  
structure (B  
**DOES NOT** form  
a single strand).



New primer **DOES**  
**NOT** hybridizes to  
the sequence B in  
the template  
nucleic acid.



Stem-loop structure is not formed (Turn  
back reaction **DOES NOT** occur).

A    B    C    D    E    F    G